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ODETE CATAPIRRA DE ALMEIDA

OCULAR MANIFESTATIONS ASSOCIATED TO ATOPIC DERMATITIS: A STUDY IN 15 DOGS

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Universidade Lusófona de Humanidades e Tecnologias

Faculdade de Medicina Veterinária

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Dissertation submitted to obtain the Degree of Master in Veterinary Medicine in the course of Master in Veterinary Medicine awarded by the Universidade Lusófona de Humanidades e Tecnologias

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DEDICATION

To my daughter Alice, for being so understanding, as a young child, for her mother's work. I dedicate this work to her for being my best friend ever, for her kindness, for her love, for her patience. And, of course, I thank you, Alice, for being so amazingly funny and amusing. When I'm before your presence, I don't need any radio or TV. Thank you for being you, as you are.

This is for your eyes only (you'll understand it, later on time):

-"*I must study politics and war, that our sons may have liberty to study mathematics and philosophy. Our sons ought to study mathematics and philosophy, geography, natural history and naval architecture, navigation, commerce and agriculture in order to give their children a right to study painting, poetry, music, architecture, statuary, tapestry and porcelain.*" Letter from John Adams to Abigail Adams (12th of May 1780).

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ABSTRACT

OCULAR MANIFESTATIONS ASSOCIATED TO ATOPIC DERMATITIS: A STUDY IN 15 DOGS

The ocular manifestations associated to atopic dermatitis, in the dog, have been described in the literature to a limited extent but a more accurate correlation between atopic dermatitis and ocular disease in the dog is required. The aim of this study is to contribute to the characterization of ocular lesions associated to canine atopic dermatitis. Ocular examinations were performed in 15 dogs that fulfilled the following inclusion criteria: diagnosis of atopic dermatitis; currently not on treatment and not previously medicated for cutaneous or ocular diseases (The CAD group). For the control group, 15 healthy dogs were selected amongst the clients of a private practice and underwent an ophthalmological exam. 40% (6/15) of the dogs in the CAD group had ocular complaint; in all the dogs with ocular complaints was found some form of ocular disease but ocular disease was also diagnosed in dogs without ocular symptoms recognized by the owner; the prevalence of ocular disease in the CAD group was 80% (12/15) being follicular conjunctivitis of the bulbar surface of the nictitans the most common ocular presentation being diagnosed in 64% (9/14) of the cases and statistically associated to the presence of CAD. Other ocular diseases were also identified, such as blepharitis and conjunctivitis (20% in both – 3/15 in both), although in a smaller number compared with the previous literature. These findings strongly support the need for an ophthalmological exam to be undertaken in all CAD patients. Further studies, with larger populations in both groups, should be undertaken in order to better characterize the clinical ophthalmic presentation of CAD.

Keywords: A*topic Dermatitis, Dog, Ocular Allergy, Lymphoid Follicular Hyperplasia, Conjunctivitis*, *Blepharitis*.

RESUMO

MANIFESTAÇÕES OCULARES ASSOCIADAS Á DERMATITE ATÓPICA: ESTUDO EM 15 CÃES

As manifestações oculares associadas com a dermatite atópica, no cão, têm sido descritas por alguns autores, embora não exaustivamente, sendo necessário o estabelecimento de uma correlação mais precisa entre dermatite atópica e doenças oculares. O objetivo deste estudo é contribuir para a caracterização de lesões oculares associadas à dermatite atópica canina. Realizou-se o exame oftalmológico num grupo de estudo de 15 cães com os seguintes critérios de inclusão: diagnóstico de dermatite atópica, atualmente sem terapêutica e não previamente medicado para doenças cutâneas ou oculares. Para o grupo controlo, foram selecionados 15 cães saudáveis aleatoriamente entre os clientes de uma clínica privada e submetidos a um exame oftalmológico. Verificou-se que 40% (6/15) dos cães do grupo com atopia apresentavam sintomatologia ocular; em todos os cães com queixas oculares foi encontrado algum tipo de doença ocular. Contudo, a presença de doença ocular também foi diagnosticada em cães onde o proprietário não reconheceu qualquer sintoma ocular. A prevalência de doença ocular no grupo de estudo foi de 80% (12/15) sendo que a conjuntivite folicular da face bulbar da membrana nictitante foi a mais comum, nomeadamente de 64% (9/14), estabelecendo-se uma relação estatisticamente significativa com a presença de atopia. A presença de blefarite e conjuntivite foram detetadas, em ambos os casos, em 20% dos cães (ambos 3/15), menor percentagem do que o anteriormente referido pela literatura. Estes resultados apoiam a necessidade de realização de um exame oftalmológico em todos os pacientes com dermatite atópica canina. Por forma a caracterizar melhor a apresentação clínica ocular da dermatite atópica no cão, deverão ser realizado mais estudos com uma maior amostragem de pacientes.

Palavras Chave: *Dermatite Atópica, Cão, Alergia Ocular, Hiperplasia dos Folículos Linfóides, Conjuntivite, Blefarite.*

ABBREVIATIONS AND SYMBOLS

AC – Allergic Conjunctivitis
AD – Atopic Dermatitis
AKC – Atopic Keratoconjunctivitis
CAD – Canine Atopic Dermatitis
CALT – Conjunctival associated lymphoid tissue
D - Diopter
DLA – Dog Leukocyte Antigen
FC – Follicular conjunctivitis
GPC – Giant Papillary Conjunctivitis
Hg - Mercurium
MALT – Mucosal associated lymphoid tissue
min - minute
mm - milimeter
NM – Nictitans membrane
OD – *Oculus Dextra*
OS - *Oculus Sinistra*
PAC - Perennial Allergic Conjunctivitis
PF - Pemphigus foliaceous
PV – Pemphigus vulgaris
SAC – Seasonal Allergic Conjunctivitis
Sd – standard deviation
STT – Schirmer Tear Test
VKC – Vernal Keratoconjunctivitis
VKH - Vogt-Koyanagi-Harada

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1. INTRODUCTION

1.1. OCULAR MANIFESTATIONS OF ATOPIC DERMATITIS IN HUMANS

Atopy is an individual personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins delivered by inhalation or ingestion, and, as a consequence, to develop typical symptoms of asthma, rhinoconjunctivitis or allergic skin disease (World Allergy Organization, 2003). Atopic dermatitis is a chronic inflammatory disease of the skin; the most common physical findings of atopy being erythematous, exudative skin lesions of the antecubital and/or popliteal skin, eyelids, corners of the mouth, neck, outer canthi, or behind the ears (Barnes, 2010; Lowery, 2012). The ocular lesions, when present, are mainly bilateral (Bonini et al., 2000) and usually relate to the different types of allergic conjunctivitis, although cataracts have been also associated with the diagnosis of atopic dermatitis; spontaneous retinal detachment is known to be more frequent in patients with atopic disease than the general population. In some advanced cases of ocular allergy, symblepharon, entropion, and trichiasis may be seen; blindness can occur as a consequence of the ocular involvement majorly when the patient develops atopic keratoconjunctivitis (Lowery, 2012). Allergic conjunctivitis has been estimated to be present in 40-60% of the allergic population (Leonardi et al. 2008).

Recently, a canine model for studying atopic dermatitis in humans was proposed, based on the similarities of cutaneous symptoms and immunological reactions (Marsella & Girolomoni, 2012).

Allergic conjunctivitis (AC) is a localized allergic condition associated frequently with rhinitis but often observed as the only or most prevalent sign of allergic sensitization (Leonardi et al., 2008). AC usually develops associated with a type I, immediate, hypersensitivity reaction which is the most common allergic response of the eye surface; this reaction can occur, in some conditions, associated with a type IV hypersensitivity reaction (Stahl & Barney, 2004; Ventocilla, 2012).

Currently in human medicine, allergic conjunctivitis is divided in 5 major subcategories: seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal keratoconjunctivitis (VKC), atopic keratoconjunctivitis (AKC) and giant papillary conjunctivitis (GPC) (Bonini, 2004; Stahl & Barney, 2004; Ventocilla, 2012). The most striking difference among this group is that SAC and PAC remain self-limiting without ocular surface damage, while VKC, AKC and GPC are vision threatening (Donshik, 1994; Stahl & Barney,

2004) It is estimated that 35% of humans develop rhinitis and conjunctivitis compared with less than 5% of dogs having canine atopic dermatitis (Marsella & Girolomoni, 2012).

It has been proposed, based on the negative results of allergic skin tests and serological antibody determinations, in the VKC and AKC, that these two diseases might represent a different model of atopy distinct from the classical type-1 hypersensitivity mechanism (Bonini, 2004; de Oliveira et al., 2007).

1.1.1. *Seasonal and perennial allergic conjunctivitis*

These conditions are commonly grouped together and are a bilateral, self-limiting conjunctival inflammatory process (Stahl & Barney, 2004). They occur through a type I hypersensitivity reaction to airborne antigens, such as pollen, grass and weeds. The manifestations are acute symptoms of ocular itching, redness, burning and tearing. They distinguish themselves as clinical entities because of the timing of the symptoms. Typically, the symptoms of SAC are seasonal and relate only to the presence of these airborne antigens. The symptoms of the PAC may last the year round and it is believed that the airborne antigens may play a role, but not exclusively, with household allergens, such as those associated with house-dust mite, cockroach dust, cigarette smoke and pet dander being more responsible for the symptoms (Stahl & Barney, 2004; Leonardi et al., 2008; Ventocilla, 2012).

The patients with SAC or PAC usually have diseases manifestating as injection of the conjunctival vessels, eyelid edema and varying degrees of chemosis. The conjunctival inflammation is quite mild and corneal involvement never occurs (Bonini, 2004). The prolongation of the symptoms through the time, might be related to a local late-phase response beginning 4 to 6 hours after allergen challenge which occurs as an increased reactivity correlating with markedly increased mast cell, neutrophil, eosinophil, basophil and macrophage infiltration of the conjunctiva (Bacon et al., 2000).

1.1.2. *Vernal keratoconjunctivitis*

VKC is a chronic keratoconjunctivitis with a type I conjunctival hypersensitivity reaction and typically occurs with exacerbation during spring months (Leonardi et al., 2008; Ventocilla, 2012) although patients may have year-round allergic symptoms (Stahl & Barney, 2004; Leonardi et al., 2006) VKC mainly affects boys in the first decade of life (Bonini et al., 2000; Leonardi et al., 2006), last 2-10 years and usually resolves during puberty (Stahl & Barney, 2004); it is a extremely rare new disease in adults (Leonardi et al., 2006). The symptoms are similar to those in SAC and PAC, although usually more severe, with photophobia, foreign body sensation and blepharospasm. Itching is the most important and

common symptom (Bonini et al., 2000; Ventocilla, 2012). Usually the eyelid skin is not involved and the lesions are bilateral, chronic and may also affect the cornea. More than 90% of the patients with VKC exhibit one or more atopic conditions, such as asthma, eczema, or seasonal allergic rhinitis (Ventocilla, 2012).

The classical pathological feature of VKC is the presence of giant papillae (> 1.0 mm) in the superior tarsal conjunctiva or in the limbus which may be associated to a thick mucoid discharge consisting of a large number of eosinophils (Belfort et al., 2000; Bonini et al., 2000; Ventocilla, 2012). These papillae are due to the overgrowth of conjunctival connective tissue, with the formation of large and sessile papillae from which overflow an abundance of collagen fibres (Leonardi et al., 2008).

The cornea may be affected with varying pathology. Punctate epithelial keratopathy may result from the toxic effect of inflammatory mediators released from the conjunctiva while shield corneal ulcers which are pathognomonic for VKC may be related to this previous pathology or, more probably, occur because of chronic mechanical irritation from the giant tarsal papillae (Ventocilla, 2012). Trantas dots occur at the limbus, these consisting of clusters of necrotic eosinophils, neutrophils and epithelial cells (Belfort et al., 2000). Keratoconus may be seen in chronic cases, probably associated to the chronic itching and rubbing of the eye (Ventocilla, 2012).

1.1.3. *Atopic keratoconjunctivitis*

Atopic keratoconjunctivitis (AKC) is a relatively uncommon but potentially blinding ocular condition (Chang-Godinich, 2012); manifesting as a bilateral, chronic inflammation of the conjunctiva and lids associated with atopic dermatitis (Bielory & Bielory, 2010, Stahl & Barney, 2004). It is estimated that AKC occurs in 20-40% of patients with atopic dermatitis and is seen in both children and adults (Bonini, 2004; Chang-Godinich, 2012) with an onset usually between 20 and 50 years (Stahl & Berney, 2004; Bielory & Bielory, 2010). In a study carried by Belfort et al., (2000), 92% of the patients with AKC reported non-ocular allergies while Moscovici et al., (2009) documented that 76.6% of the patients withf atopic dermatitis in his study population had AKC.

Unlike VKC, atopic keratoconjunctivitis is generally perennial, although an aggravation of the condition during the winter months is often seen. AKC may affect the eyelid skin and lid margin, conjunctiva, cornea, and the lens, although there is some speculation that the long-term use of topical corticosteroids can induce the lenticular changes later in life rather than the inflammatory disease itself (Bielory & Bielory, 2010; Ventocillo, 2012). Typically VKC has a chronic presentation, with itching as the most important symptom (Belfort et al., 2000). The corneal involvement is usually represented by punctate epithelial

keratopathy, varying degrees of corneal neovascularization, stromal scarring and possible ulceration (Belfort et al., 2000; Ventocillo, 2012); Trantas dots at the limbus are also a common sign (Chang-Godinich, 2012). Fibrosis or scarring of the conjunctiva may result in symblepharon while chemosis can also occur and the conjunctiva usually shows a typical papillary reaction, more prominent in the inferior tarsal quadrant, in contrast to that seen in VKC (Bonini, 2004; Ventocillo, 2012). Although the presence of excessive mucin may be seen (Chang-Godinich, 2012), the ocular discharge is usually watery and clear, not mucoid (Ventocillo, 2012). Hypersensitivity type I and IV reactions are involved in the pathogenesis of these ocular inflammatory changes in the AKC (Chang-Godinich, 2012).

A more severe presentation usually involves the presence of typical shield-shaped corneal ulcers, keratoconus and posterior/anterior subcapsular shield-shaped cataracts (Chang-Godinich, 2012). Other complications associated to AKC are ocular herpes simplex infections and secondary staphylococcal blepharitis (Bielory & Bielory, 2010).

1.1.4. *Giant papillary conjunctivitis*

Giant papillary conjunctivitis (GPC) is an immune-mediated inflammatory disorder of the superior tarsal conjunctiva with the primary findings being the presence of giant papillae on the tarsal conjunctiva of the upper eyelids (Stahl & Barney, 2004); these papillae are typically greater than 0.3 mm in diameter (Venticello, 2012). The use of contact lenses is usually associated with this condition although a variety of foreign bodies can also be involved, such as exposed sutures and ocular prostheses (Venticello, 2012). The initiating event is believed to be mechanical irritation and/or antigenic stimulus of the tarsal conjunctiva of the upper lids, perhaps by a contact lens surface or edgeof both rigid and flexible lenses or a deposit on the lens surface itself (Weissman, 2011), followed by a hypersensitivity type I reaction. Heat sterilization, poor cleaning, rough contact lens edges, and extended wearing times favor the development of GPC (Weissman, 2011) with the use of hydrogel contact lenses appearing to predispose to this condition with a larger prevalence (20%) than that seen with the use of rigid lenses (5%) (Donshik, 1994). The GPC is usually a benign condition and after appropriate treatment, 90% of patients can usually return to comfortable contact lenses wear (Donshik, 1994; Weissman, 2011).

1.2. CANINE ATOPIC DERMATITIS

1.2.1. *Introduction*

Canine atopic dermatitis (CAD) was first described as a clinical entity by Halliwell et al., in 1971; Patterson (1959) published the first case report of CAD in a dog that was allergic

to ragweed. CAD is defined, by the American College of Veterinary Dermatology (ACVD) task force on CAD as “a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens” (Olivry, DeBoer & Griffin, 2001; Halliwell, 2006). More recently, Favrot et al., (2010) included the food allergens as additional cause of CAD by proving that food-induced atopic dermatitis and non-food induced atopic dermatitis were clinically indistinguishable.

The prevalence of CAD is estimated to be between 3 and 15% although the true prevalence and incidence of CAD remains unknown and varies between dogs of different breeds and in different environments. It is considered the second most common cause of canine pruritus, after the flea allergy dermatitis (Hillier & Griffin, 2001). The difficulties in establishing a true prevalence are mostly due to under diagnosis, due to the mild manifestation of the disease in most dogs, with control by symptomatic medication. In such cases a definitive diagnosis is rarely reached and other manifestations of the disease, such as chronic otitis and cutaneous infections are rarely documented (Hillier & Griffin, 2001).

Although considered a genetically inherited disease, as in humans, atopy is considered to be multifactorial (Sousa & Marsella, 2001) and it is only recently that 11 genes were identified to be altered in atopic patients in comparison to healthy dogs (Wood et al., 2009).

There is an abundance of clinical evidence implying that atopic dermatitis is antigen driven, through the interaction of allergen-specific IgE with the relevant allergen being the main mechanism in atopy (Halliwell & De Boer, 2001). Numerous environmental allergens have been incriminated in the pathogenesis of CAD, such as dust and storage mite antigens, house dust, pollens from grasses, trees and weeds, mould spores, epidermal antigens, insect antigens, and miscellaneous antigens such as kapok (Hill & DeBoer, 2011). Food allergens, as previously mentioned, should be included as a potential cause or contribute for the development of CAD (Olivry, 2007; Favrot, 2010).

The typical age of onset of this disease in dogs is between 6 months and 3 years and there are several breeds documented to be at higher relative risk including the Beauceron, Boston terrier, Boxer, Cairn terrier, Chinese shar pei, Cocker spaniel, Dalmatian, English bulldog, English setter, Fox terrier, Irish setter, Labrador retriever, Labrit, Lhasa apso, Miniature Schnauzer, Pug, Scottish terrier, Sealyham terrier, Setter, West Highland White terrier, Wire-haired Fox terrier and Yorkshire terrier (Griffin & DeBoer, 2001). There appears to be no sex predisposition and the manifestations can be seasonal or not, depending upon the allergens involved. Initial signs are reported to be seasonal in 42-75% of

the dogs; primary lesions of CAD, when present, are erythema and pruritus. Generalized pruritus is reported in 40% or more of the dogs; when localized, the dog exhibits pruritus of the face, ears, paws, extremities and ventrum. Combinations of pruritus of any of these areas can be noted (Griffin & DeBoer, 2001). Secondary lesions are common and include red-brown salivary staining from behaviour such as foot sucking, excoriations, self-induced alopecia, dry lusterless hair, hyperpigmentation, scaling and lichenification; acute moist dermatitis, acral pruritic nodules and bacterial pododermatitis. Seborrhea and hyperhidrosis are also described as complications of CAD. Otitis externa (seen in 86%) and conjunctivitis (noted in 50%) are also reported to be very commonly associated with CAD (Griffin & DeBoer, 2001).

1.2.2. *Diagnosis*

There is no single feature or symptom in CAD allowing a definitive diagnosis upon physical examination or clinical history of the patient. The diagnosis of this condition is difficult because none of the typical signs is pathognomonic (DeBoer & Hillier, 2001[a]; Favrot et al., 2010). The issue of disease definition is considered of crucial importance, not only for the establishment of a correct diagnosis, but also for enrolment of a homogeneous population of subjects in clinical trials or for the collection of samples for research studies (Olivry, 2010). Willemse (1986) and Prélaud et al. (1998) established the first lists of clinical criteria attempting to define uniform clinical criteria for diagnosing CAD (DeBoer & Hillier, 2001[a]). Recently, Favrot et al. (2010), showed these criteria to have, respectively, a sensitivity of 49-74% and a specificity of 68-80%. The same author recently described a new criteria list for clinical diagnosis with a sensitivity of 85.4% and specificity of 79.1% (with five positive criteria) (table 1) - The International Task Force on Canine Atopic Dermatitis supports the recommendation of using this parameters in general practice as well as their use for the homogeneity of enrolment of canine subjects in research studies (Olivry, 2010). This is considered to be adequate for a screening test, not to be used alone but in the context of a thorough clinical examination after exclusion of ectoparasites, fungal and bacterial infections (Favrot et al., 2010; Olivry, 2010).

In addition and before allergy testing, an elimination diet should be carried out to determine whether food allergens play a role in the development of the disease (Olivry, 2007; Favrot et al., 2010). As a final step, once the clinician considers that CAD is probable, allergy tests may be conducted to provide additional evidence to substantiate the diagnosis (DeBoer & Hillier, 2001[a]).

Table 1 - The 2010 Favrot Diagnostic Criteria for Canine Atopic Dermatitis (Favrot et al., 2010).

1.Onset of signs under 3 years of age
2.Mostly indoors
3.Corticosteroid- responsive pruritus
4.Pruritus sine material at-onset
5.Affected from feet
6.Affected ear pinnae
7.Non-affected ear margins
8.Non-affected dorso-lumbar area

There are two methods for allergy testing in veterinary medicine: the serum measurement of allergen-specific IgE and the intradermal testing; both demonstrate which aeroallergens the atopic dog is hypersensitive (DeBoer & Hillier, 2001[b]; Hillier & DeBoer, 2001).

The histopathology of skin lesions, although not routinely performed as a tool for the establishment of the definitive diagnosis might be useful since canine atopic skin lesions have been characterized as exhibiting an inflammatory pattern characterized as a chronic, hyperplastic and spongiotic, mixed perivascular dermatitis (Olivry & Hill, 2001).

1.3. CANINE DERMATOLOGY IN OPTHALMOLOGY

1.3.1. *Blepharitis*

Blepharitis is the inflammation of the eyelids (Scott, Miller & Griffin, 2001[b]) and refers to a number of inflammatory conditions, with the primary cause often being masked to some extent by possible secondary complications (Bedford, 1999). The term is also used to describe inflammation of the eyelids that involve the meibomiam glands (Bistner, 1994). Pain is indicated by blepharospasm and excessive lacrimation with epiphora, which may be worsened by self-trauma (Stades & Gelatt, 2007). Associated conjunctivitis or keratoconjunctivitis is not an uncommon finding; cicatrization and lid distortion may be sequelae of chronic blepharitis. The clinical presentation may be focal or diffuse, unilateral or bilateral and may involve the upper or lower eyelids (or both) (Gelatt, 1991).

1.3.1.1. Chalazion and Hordeolum

A chalazion is an eyelid granuloma which results from retention of glandular secretions in the meibomian gland due to inflammatory or non-inflammatory cicatrization of the meibomian gland duct (Gellat, 1991). The oily material from the gland extrudes into the

lid tissue and acts as a foreign body eliciting a granulomatous reaction (Bistner, 1994). The diagnosis is clinical, through the identification of a yellow, firm and elevated mass in the conjunctival surface of the eyelid (Gellat, 1991). This inflammation may predispose to a local staphylococcal infection and thus hordeolum formation (Stades & Gellat, 2007).

An hordeolum is a suppurative inflammation of the meibomian (internal hordeolum) or Zeiss and Moll glands (external hordeolum) usually due to staphylococcus infection. The external presentation tends to occur mainly in young animals while the internal hordeolum is more recognized in middle-age adults (Gelatt, 1991). Both have a clinical diagnosis through the visualization of a single or multiple focal abscesses on the external or inner surface of the eyelids (Bedford, 1999; Stades & Gelatt, 2007). The affected lids are usually swollen and painful (Stades & Gelatt, 2007).

1.3.1.2. Meibomianitis

Meibomianitis is the inflammation of the lids that involves the meibomian glands and can produce a severe form of marginal blepharitis especially in young dogs (Bistner, 1994). In puppies, can occur as a purulent blepharitis as part of juvenile cellulitis (eg: "puppy strangles", “head-gland disease” or "juvenile pyoderma") (Bedford, 1999; Almeida & Sousa, 2001; Neuber et al., 2004; Basset et al., 2005). It has been recently renamed by Peña & Leiva (2008) as necrotizing marginal blepharitis; the pathogenic mechanism is thought to be related to the presence of *Staphylococc*al bacteria and the immune-mediated reaction induced by staphylococcal toxins (Chambers & Severin, 1984).

The primary clinical lesions of juvenile cellulitis seen in affected dogs are pustular moist dermatitis of the head and face with furunculosis and mandibular and prescapular lymphadenopathy (Mason & Jones 1989; Reimann et al., 1989), although purulent otitis (Almeida & Sousa, 2001; Hutchings, 2003; Neuber et al., 2004) and fistulous drains and furunculosis of the back and loin can also be observed (Almeida & Sousa, 2001). Systemic illness is rarely seen and the lesions are microscopically a pyogranulomatous inflammation (Reimann et al., 1989). When associated with this condition, meibomianitis is clinically diagnosed through the direct observation of the swollen and often enlarged meibomian glands in the conjunctival surface of the eyelid (fig.1). The lid is usually swollen, red, pruritic and painfull (Stades & Gellat, 2007; Peña & Leiva, 2008). The complications of meibomianitis include the formation of chalazia and reduction or loss of the lipid layer of the precorneal tearfilm (Bistner, 1994; Stades & Gellat, 2007).

The exact cause of canine juvenile cellulitis and eyelid involvement are unkown but a bacterial hypersensitivity has been postulated to explain the response to corticosteroids and the explosive course of the disease (Cullen & Webb, 2007) although in only a few cases

a bacterial association has been definitively established (Reimmann et al., 1989; White et al., 1989; Hutchings, 2003; Neuber et al., 2004).

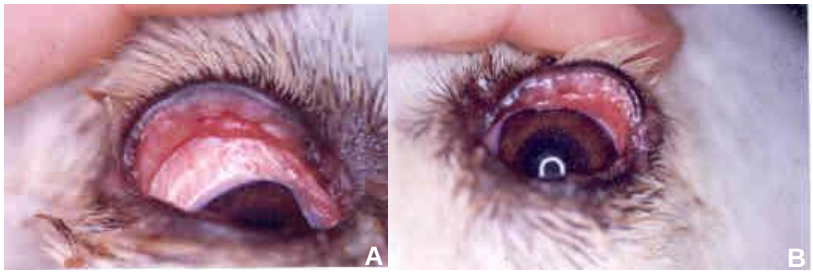


Figure 1 (A and B) – Meibomianitis in both eyes (A and B). This dog also had juvenile cellulitis.

1.3.1.3. Bacterial blepharitis

Staphylococci and *Streptococci* spp. are most commonly involved in bacterial blepharitis (Gerding et al, 1988; Stades & Gellat, 2007). These bacteria are the most commonly isolated from conjunctival tissue in normal and inflamed eyelids (Urban et al., 1972; Murphy et al., 1978); infectious blepharitis occurs when animals develop bacterial infection at the base of the lash follicles as well as in the meibomian glands. The lid margins become swollen, reddened, and inflamed, and the affected animal rubs the face and eyes (Bistner, 1994). Over several weeks, ulceration of the eyelid skin and margins and other complications such as alopecia and fibrosis may develop (Stades & Gellat, 2007). Conditions that predispose to ocular irritation such as trichiasis of facial hair and atopic dermatitis, may also predispose to bacterial blepharitis (Bistner, 1994; Whitley, 2000). Staphylococcal infections may also cause eyelid multiple pyogranulomas (Stades & Gellat, 2007) although Sansom et al. (2000) were not able to identify any bacteria in pyogranulomatous blepharitis in one dog which responded to cephalexin treatment. The diagnosis of bacterial blepharitis should be supported by identification of the agent as well an attempt to identify an underlying cause should be made.

1.3.1.4. Mycotic blepharitis

Primary mycotic blepharitis involving fungi other than the dermatophytes is uncommon (Gelatt, 1991). Infection with *Microsporum* and *Trichophyton* sp. is seen as part of a generalized problem in young dogs (Bedford, 1999); rarely, however, are only the lids involved (Gelatt, 1991). The lesions may consist of any combination of papules, pustules, focal to wide spread areas of alopecia, variable erythema, and variable scaling and crusting. Kerion reactions (nodular lesions), particularly on the face, may mimic areas of deep pyoderma and/or furuncolosis or even autoimmune diseases (Moriello, 2004). Diagnosis is confirmed by staining skin scrapings with either Gram or Giemsa stain (Stades & Gellat,

2007), by direct examination of hairs and scales on the optic microscope (Moriella, 2004) or by culturing the organisms on Sabouraud's agar or dermatophyte test medium (Stades & Gellat, 2007); fungal culture is considered the "gold standard" for diagnosis (Moriella, 2004).

1.3.1.5. Parasitic Blepharitis

Parasitic involvement of the eyelids is usually a part of a generalized process in the demodectic and sarcoptic mange with the lesions being characterized by alopecia with varying degrees of hyperemia and pruritus (Gelatt, 1991) as well as often being complicated by secondary bacterial infection and self-trauma (Bedford, 1999). Infections of the eyelids associated to systemic infection by the protozoan *Leishmania infantum* should also be considered as an important differential in endemic countries or in dogs that have travelled to an endemic country, as a cause of parasitic blepharitis (McConnel, 1970; Roze, 1986; Molleda et al., 1993; Roze, 1986; Koutinas et al., 1999; Peña et al., 2000) since it described by Roura et al. (1999) that *Leishmania* organisms can actually be present in the skin inflammation of infected individuals.

Demodectic mange

Demodex mites are considered to be a normal part of the cutaneous microfauna in the dog; it is assumed that immunosuppression or a defect in the skin immune system allows mites to proliferate in hair follicles, resulting in clinical signs (Mueller et al., 2012). In young dogs with localized lesions, the disease tends to be restricted to the face, where eyelid involvement is commonplace (Bedford, 1999). Typical lesions are erythematous and alopecic patches (Mueller, 2004). The diagnostic test of choice is multiple deep skin scrapings of the affected skin; trichograms are particularly useful in areas that are difficult to scrape such as periocular and interdigital areas (Mueller et al., 2012).

Sarcoptic mange

Sarcoptic mange is a highly contagious pruritic skin condition caused by infestation with the mite *Sarcoptes scabiei*. In dogs, the disease typically presents as an intensely pruritic, papulocrustous dermatosis affecting the periocular skin, pinnal margins, elbows and hocks, which in time may generalize (Curtis, 2004). Recently, a localized form of sarcoptic mange was demonstrated in 10 dogs where in one dog the localized area was the right periocular region in addition to the ipsilateral pinna (Pin et al., 2006). The definitive diagnosis relies on the demonstration of mites and/or their eggs. The tests, namely skin scrapings, dermatohistopathology and faecal flotation, are however relatively insensitive; a commonly

used diagnostic “test” is the therapeutic trial (Curtis, 2004) since a response to therapy, such as decrease in pruritus, usually implies that the diagnosis was correct (Fourie et al., 2010).

Leishmania blepharitis

Leishmania blepharitis, as previously mentioned, is described as occurring in 26% of the dogs with leishmaniasis caused by *Leishmania infantum* (Peña et al., 2000) or in 34% of affected dogs, in a study undertaken by Molleda et al. (1993). The clinical presentation of blepharitis associated with *Leishmania* infection was variable: Peña et al. (2000) noted periocular alopecia (lunettes); a diffuse nodular form with generalized edema, induration and hyperemia of the eyelids; an ulcerative form; and in a small number of dogs, a solitary nodule. Recently, was suggested that granulomatous myositis of the various eyelid muscles, cause by the presence of the parasite, can be implicated for the development of clinical blepharitis (Naranjo et al., 2010). Cutaneous signs of leishmaniasis in dogs are the most common lesions (Sollano-Gallego, 2009) and are often characterized by a dry, seborrheic dermatitis, desquamation, and associated alopecia (Slappendel, 1988; Ciaramella et al., 1997; Koutinas et al., 1999; Peña et al., 2000) as well as skin ulceration (Koutinas et al., 1999). In a recent study in Portugal, cutaneous lesions were described in 40% of the animals (Oliveira et al, 2010). Being a systemic disease, besides the dermatologic and ocular signs, generalized lymphadenomegaly, progressive weight loss, muscular atrophy, exercice intolerance, decreased appetite, lethargy, splenomegaly, polyuria and polidipsia, epistaxis, onychogryphosis, lameness, vomiting and diarrhea are also common signs (Solano-Gallego et al., 2009) The diagnosis of canine leishmaniosis is made by detection of specific and quantitative serum anti-leishmanial antibodies and/or demonstration of the parasite in tissues by applying molecular techniques (Solano-Gallego et al., 2009; Solano-Gallego & Cardoso, 2009). In dogs with clinical signs and/or clinicopathological abnormalities consistent with leishmaniosis, the diagnostic methods also include the detection of amastigotes in stained cytological smears of aspirates from cutaneous lesions, lymph nodes, bone marrow and spleen (Solano-Gallego & Cardoso, 2009).

1.3.1.6. Immune-Mediated Blepharitis

The immune-mediated blepharitis is commonly associated with 3 types/groups of diseases: pemphigus group, medial canthal ulcerative blepharitis and the Vogt-Koyanagi-Harada – *like syndrome*, or uveodermatological syndrome (Bedford, 1999; Stades & Gellat, 2007; Peña & Leiva, 2008).

Pemphigus complex

In dogs, there are five described varieties - foliaceus, eryrhematosus, vulgaris, vegetans, and bullous (Marsella, 2000; Scott et al., 2001[a]). To our knowledge, only the first three types of pemphigus are scientifically reported with eye including lesions (Stades & Gellat, 2007; Peña & Leiva, 2008).

The pemphigus group of vesiculobullous epidermal diseases can involve mucocutaneous junctions, where eyelid inflammation and ulceration is commonly seen (Stades & Gellat, 2007; Peña & Leiva, 2008). Autoimmunity toward keratinocyte-keratinocyte adhesion molecules induces epidermal acantholysis characterist of pemphigus (Marsella, 2000; Olivry & Chan, 2001).

Pemphigus foliaceous (PF) is the most frequent manifestation of the pemphigus complex and clinically affects the skin of the face (where periocular lesions are considered amongst dorsal muzzle, head and/or preauricular regions) in 82,5% of the cases. This disease is also reported to affect the pinna, dorsum, ventrum, feet and limbs (Olivry & Chan, 2001; Vaughan et al., 2010). The skin lesions are comparable in most canine patients: transient vesicles and pustules evolve rapidly into erosions and crusts; a remarkable finding of PF is the predilection of skin lesions for the footpads (Olivry & Chan, 2001). Pemphigus erythematous also affects the face and lids as the previous one (Gonsalves-Hubers, 2005) as ulcerative and scaly lesions (Scott et al., 2001[a]). Pemphigus vulgaris (PV) is the most severe type of pemphigus, with a systemic involvement of the oral cavity, nail beds, and skin and mucocutaneous junctions of the eyelids, lips, external nares, and ears (Bedford, 1999; Olivry & Chan, 2008). The skin lesions consist of erythema, erosions, ulcerations, scales, and crusts (Olivry & Chan, 2001). The diagnosis of the different kind of pemphigus is based on compatible history, clinical signs and skin histopathology (Peña & Leiva, 2008).

Medial canthal ulcerative blepharitis

Medial canthal ulcerative blepharitis represents a juxta-palpebral disorder, usually affecting the medial canthus (Stades & Gellat, 2007; Peña & Leiva, 2008). Clinically corresponds to slightly thickened, hairless, pinkish, non-pruritic defects located 5-10 mm from the margin of the lid. They spread very slowly and are probably similar to the pemphigus group, but usually without defects elsewhere (Stades et al., 1998). The condition is usually bilateral and the histopathological exam allows the differentiation from the pemphigus group. The German shepherd, Long-Haired Dachshund, and Toy and miniature Poodle are the most affected breeds (Stades & Gellat, 2007; Peña & Leiva, 2008).

Acute allergic blepharitis

Acute allergic blepharitis can occur at any age and in atopic or non-atopic dogs (Peña & Leiva, 2008) and is characterized by an-acute onset edema and hyperemia. Atopic blepharitis may result from local exposure to a contact allergen or occur as a part of a more generalized immune response (Bedford, 1999; Stades & Gellat, 2007). Swelling of the eyelids and muzzle will be seen following insect bites (ants, ticks, fleas) and postvaccinal reactions (Stades & Gellat, 2007; Peña & Leiva, 2008). The condition manifests itself with intense itching, eye redness, and dramatic and immediate swelling of the eyelids and conjunctiva which may be so severe that the eye closes (Peña & Leiva, 2008). Usually this condition is self-limiting and while the diagnosis is made through clinical examination; it is of importance to compile a completed history taking in consideration environmental aspects such as recent exposition to cut grass, plant pollen allergens in the surroundings, cleaning products, and so on (Peña & Leiva, 2008).

Acute angioneurotic edema

Another type of acute blepharoconjunctivitis allergy involves the urticarial lesions of acute angioneurotic edema (Peña & Leiva, 2008). Lesions are characterized by the acute onset of edema of the skin and subcutaneous connective tissue of these areas. Swelling around the eyes may be severe enough to close the orbital fissures and prevent the animal from seeing (Bistner, 1994). The cause of urticarial eye disease is usually associated with the stings of insects, with ingestion of spoiled protein material in foods, or with the administration of systemic drugs against which the animal has an allergic reaction (Peña & Leiva, 2008).

Vogt-Koyanagi-Harada-*like syndrome*

Vogt-Koyanagi-Harada-*like syndrome* or uveodermatological syndrome is a condition where usually the first complaint of the owner is the depigmentation of the nose and eyelids (Stades & Gellat, 2007; Peña & Leiva, 2008). It will be further discussed in section 1.3.3.

1.3.2. *Conjunctivitis*

Conjunctivitis is defined as an inflammation of the conjunctiva and can be a primary condition or secondary to other ocular or systemic disorders. Clinically conjunctivitis is recognized as a red and hyperemic conjunctiva which may or not be associated with conjunctival oedema and/or a lymphoid follicular hyperplasia (Gelatt, 1991); in this later case is usually termed follicular conjunctivitis (Ward, 1999). Chemosis, hyperemia, and cellular

exudation generally characterize acute conjunctivitis (Hendrix, 2007). Chronically the condition occurs in association a keratinization of the epithelial cells of the conjunctiva (Hendrix, 2007).

Follicular conjunctivitis most frequently involves the bulbar aspect of the nictitans membrane (NM) and is characterized by an increase of number and size of the conjunctival lymphoid follicles, and is often associated with conjunctival hyperemia as well as a mucoid ocular discharge (Ward, 1999). The presence of a small number of lymphoid follicles, closely associated with the NM gland is considered normal in the canine patient (Ward, 1999).

There are several causes of conjunctivitis (bacterial, viral, fungal, rickettsial, parasitic, tear deficiency, ligneous conjunctivitis, allergy, chronic antigenic stimulation) (Hendrix, 2007); we will only describe the ones that are associated or might be clinically associated to CAD such as allergic conjunctivitis (Hendrix, 2007) and follicular conjunctivitis (Lourenço-Martins et al., 2011).

1.3.2.1. Allergic conjunctivitis

Allergic conjunctivitis (AC) is described as a frequent occurrence in the dog and as a common component of CAD (Hendrix, 2007); the most common allergens are pollens, dust and bacterial toxins (Bedford, 1999; Hendrix, 2007). The clinical manifestations are hyperemia and chemosis of the conjunctiva (fig. 2) with intense pruritus that causes further blepharoedema (Martin, 1973; Bedford, 1999; Hendrix, 2007). A serous ocular discharge is often present as well, and with chronic stimulation conjunctival follicles develop (Bedford, 1999; Hendrix, 2007). The affected animals present with chronic epiphora and ocular redness without any other ophthalmic signs (Peña & Leiva, 2008). Results of cytology and histopathology performed on conjunctival scrapings and biopsy specimens can suggest the presence of an allergic response (Hendrix, 2007). Allergic conjunctivitis is characterized cytologically by the presence of eosinophils, basophils and lymphocytes (Brooks, 1991); finding one eosinophil on cytological examination of a conjunctival scraping is considered to be diagnostic for an allergic process (Lavach et al., 1977; Brooks, 1991; Hendrix, 2007). The diagnosis can be challenging; the conjunctival biopsy shows mild lymphoplasmacytic infiltrate with variable numbers of eosinophils, vascular congestion, and dilation (Peña & Leiva, 2008).

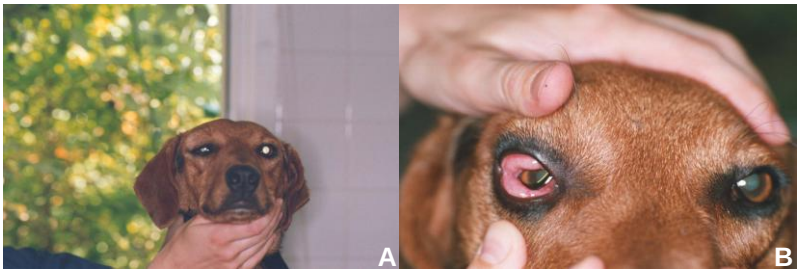


Figure 2 (A and B) – Allergic conjunctivitis (acute). Note the blepharospasm in 2 – A and the intense chemosis in 2- B.

1.3.2.2. Follicular conjunctivitis

Follicular conjunctivitis (FC) consists of a macroscopic proliferation of the conjunctival-associated lymphoid tissue of the palpebral or bulbar conjunctiva (Peña & Leiva, 2008). It is thought to develop secondary to chronic antigenic stimulation (Bedford, 1997; Hendrix, 2007). At first, was included as a syndrome under viral etiologies prematurely (Martin, 1973; Jackson & Corstvet, 1975) but it has been demonstrated by Jackson & Corstvet (1980) that follicle formation is not secondary to bacterial or viral causes. The follicles form in FC primarily on the bulbar surface of the nictitans, but they may also form elsewhere in the conjunctiva (Bedford, 1999). These follicles greatly outnumber those normally seen and they can be significantly larger as well (Bedford, 1999). Frequently, hyperemia of the conjunctiva is involved and a mucoid ocular discharge is seen in association with FC (Bedford, 1999; Hendrix, 2007). This condition is described as occurring more frequently in dogs younger than 18 months of age (Bedford, 1999; Hendrix, 2007) although older animals can also be affected (Peña & Leiva, 2008).

The diagnosis is made by clinical signs and conjunctival cytology which demonstrate the presence of lymphocytes (Peña & Leiva, 2008). Clinical and specially when affecting the nictitans membrane, this condition should be differentiated from the plasmacytic conjunctivitis of the nictitans; this later is a chronic inflammatory condition of the exposed areas of this membrane characterized by raised pink-head sized non-pigmented lesions near the free margin of the anterior surface of the nictitans (Read, 1995).

1.3.3. *Uveitis*

There are several systemic diseases that result in periocular dermatological signs associated with uveitis, such as leishmaniasis, rickettsial diseases, ehrlichiosis, systemic lupus erythematous, amongst others (Nell, 1997). We will only describe the Vogt-Koyanagi-Harada-*like syndrome* (VKH-*like syndrome*), or uveodermatologic syndrome since it is clearly, to our knowledge, the only disease with clear association between dermatological and ocular signs; the VGT – *like syndrome* is a rare canine condition, occurring mostly bilaterally, although recently a unilateral presentation was described by Sigle et al. (2006).

1.3.3.1. Vogt-Koyanagi-Harada-*like syndrome*

The VKH-*like syndrome* has been described in purebred dogs including the Akita (Cottrell & Barnett, 1987; Lindsley et al., 1991; Murphy et al., 1991; Pye, 2009), Golden Retrivier, Old English Sheepdog, Shetland Sheepdog, Saint Bernard, Samoyed, Irish Setter, Australian Sheperd (Bistner, 1994), Siberian Husky (Bistner, 1994; Sigle et al., 2006) Dacshund (Herrera & Duchene, 1998), Beagle, Chow chow (Cullen & Webb, 2007; Peña &

Leiva, 2008) and Brazilian fila dog (Laus et al., 2004). In humans is described as an immune-mediated disease in which melanocytes are targeted (Morgan, 1989; Peña & Leiva, 2008) and in dogs Carter et al. (2005) found immunohistochemical observations that also supports this mechanism of disease. The causes responsible for the development of cellular hypersensitivity to melanin have not been elucidated, although specific circulating anti-melanin autoantibodies and melanin-sensitized lymphocytes have been reported in affected patients (Peña & Leiva, 2008). A genetic basis seems to be involved; Angles et al. (2005) identified differences in the Dog Leukocyte Antigen (DLA) in the Akita breed that seems to contribute for the pathogenesis of the disease in this breed. Dogs are typically affected in adulthood, and ocular lesions usually precede the dermatologic lesions, which are localized in the mucocutaneous junctions (fig.3) (Morgan, 1989; Peña & Leiva, 2008). Clinically, in the dog, is recognized as dermal depigmentation involving the eyelids, nose, lips, pads of the feet, scrotum, and the anus (Bistner, 1994). Often, loss of pigmentation of the eyelids and nose is the primary clinical sign recognized by the owner (Peña & Leiva, 2008) although they can be presented as well for a complaint of sudden blindness or gradual vision loss (Cullen & Webb, 2007). Ocular lesions vary from bilateral anterior uveitis to severe panuveitis (Morgan, 1989) characterized by iridal or choroidal depigmentation (Townsend, 2008). Bullous retinal detachments may occur, and secondary cataracts and glaucoma are common (Cullen & Webb, 2007). Dermal and hair depigmentation develop either gradually or rapidly, and they may be ulcerative on nature (Cullen & Webb, 2007). Alopecia is an inconsistent finding (Morgan, 1989) and neurologic involvement is rare (Cottrell & Barnett, 1987).

The diagnosis is made by means of clinical signs (fig. 3) and histopathological examination of skin biopsies (Peña & Leiva, 2008). At present, there is no specific diagnostic test for VKH-*like syndrome in dogs* (Cullen & Webb, 2007).

Figure 3 – Vogt-Koyanagi-Harada-*like syndrome*. In this figure is observed the typical loss of pigmentation around the eyelids and nose (photo courtesy of Dr. David Williams).

1.3.4. *Ocular manifestations and canine atopic dermatitis*

Ocular allergy usually refers to conditions affecting the lids, conjunctiva and cornea; it might often be present in conjunction with dermatologic signs and should be considered in animals where itching and pawing the eyes (Bistner, 1994) is a predominant symptom, without the clinical evidence of foreign bodies and/or another causes of ocular pain. Atopy or clinical allergy to environmental antigens involves the face in approximately 80% of cases, and 50% have conjunctivitis (Martin, 1999).

More chronic allergic reactions are seen in atopic animals during prolonged exposure to the sensitizing agent. The conjunctival vasculature becomes congested, and there may be chemosis and a serous discharge. The eyes are pruritic and the affected animal rubs at them with the paws or rubs the face across the carpet or furniture. This chronic irritation produces further excoriation of the lids. In protracted cases, the conjunctiva becomes markedly thickened and there is a heavy seromucoid discharge. Keratitis may be a complication of chronic atopic conjunctivitis. Corneal involvement can range from superficial vascularization to epithelial erosions (Bistner, 1973).

There is little in the literature describing the ocular manifestations of canine atopic dermatitis, although recently, Lourenço-Martins et al. (2011), found that conjunctival hyperemia was the most common sign of ocular allergy associated to CAD occurring in 90% of the 60 cases in his study, followed by pruritus (73%), chemosis (70%), ocular discharge (60%), epiphora (57%) and corneal involvement (10%). In the same study, was found a prevalence of 60% of allergic conjunctivitis and a significantly correlation between severity of ocular lesions and ocular pruritus as well as to skin lesions score for head region (Lourenço-Martins et al., 2011). In cases of allergic conjunctivitis, they document signs of: epiphora, conjunctival hyperemia, squinting and a certain degree of blepharospasm in acute cases, and in chronic cases the study reported follicular conjunctivitis with hypertrophy of lymphoid follicles of the bulbar conjunctiva and inner part of the nictitans and the presence of a white mucous discharge (Lourenço-Martins et al., 2011). These later conditions had already been suggested by Bistner (1994) that when associated with rubbing the eyes might have other signs of dermatologic irritation, such as atopic dermatitis.

Furiani et al., (2011) describes that bacterial colonization of the conjunctival sac in atopic dogs is more frequent and greater in extent than in healthy dogs. The high prevalence of bacterial colonization in the conjunctival sac of atopic dogs seems to reflect the elevated bacterial colonization observed in the skin of such patients, where *S. pseudointermedius* is the most frequent species isolated. Lymphocytes and eosinophils were also more frequent in conjunctival cytology from atopic dogs (Furaini et al., 2010).

Bistner (1994) also describes clinically atopic blepharitis and conjunctivitis in the dog, but unfortunately, he mainly based he's comments on human medicine literature limiting the use of his description as valid canine clinical models for symptoms. Peña & Leiva (2008) describe the ocular manifestations associated to canine atopy as a chronic blepharitis and conjunctivitis characterized by redness, blepharospasm, erythema, and crusting extending from the eyelid margin upward for 8 to 10 mm accompanied by excoriation and ulceration. However they consider canine food hypersensitivity as a different entity from atopy; the ocular lesions associated are pruritus and a variety of primary and secondary skin lesions including: papules, plaques, pustules, wheals, angioedema, erythema, ulcers, excoriation, lichenification, pigment changes, alopecia, scales, crusts, and moist erosions.

1.4. OBJECTIVES

The aims of this study are: to contribute, preliminary, to the knowledge and characterization of ocular lesions associated to canine atopic dermatitis diagnosed based on the more recent criteria previously mentioned in section 1.2.2.; to demonstrate the need to perform an ocular examination in these patients.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. *Equipment*

The dogs were examined on an examination table in a darkened room. Ophthalmic examination was achieved using a Finnoff transilluminator (Welch Allyn®; Skaneateles Falls, USA) to aid inspection of general aspects of the globe and adnexae, followed by direct ophthalmoscopy using a direct ophtalmoscope (Welch Allyn®; Skaneateles Falls, USA) and indirect ophthalmoscopy after pupil dilation with tropicamide 1% - Tropicil Top® (Edol; Linda-a-Velha, Portugal) using a 20 D lens (Welch Allyn®; Skaneateles Falls, USA) and indirect ophthalmoscope (Welch Allyn®; Skaneateles Falls, USA). A Slit Lamp Biomicroscope Kowa SL – 15 (Kowa®, Tokyo, Japan) was used to observe the anterior segment of the eye, to evaluate the pupillary and Dazzle reflexes and as well as to assess the presence of a Tyndall effect for flare in the anterior chamber. Schirmer Tear Test (Eickemeyer®; Ehastraße, Germany) for measuring aqueous lachrymal production; 0.4% oxibuprocain – Anestocil® (Oftalder; Oeiras, Portugal) to anesthetize the nictitans surface before its inspection and the cornea before measuring the intra-ocular pressure. Evaluation for corneal ulceration employed fluoret strips (Haag-Streit International®; Koeniz, Switzerland) while measurement of intraocular pressure used a Tonopen XL (Medtronic Solan®; Jacksonville, Florida; USA) with Ocu-film Tip Covers (Reichert®; Ney York, USA) to cover the sensor surface. To wash the ocular surface as needed, a sterile isotonic saline solution (NaCl 0.9%, B.Braun Medical, Barcarena, Portugal) was used.

2.1.2. *Population*

Our study population comprised the atopic dogs presented at the Dermatology Service of Small Animal Hospital of Faculdade de Medicina Veterinária – Universidade Lusófona de Humanidades e Tecnologias (FMV-ULHT). We selected 15 patients that matched the following criteria: the animals had a diagnosis of atopic dermatitis according to Favrot et al., 2010; and were not receiving systemic or ocular medication. They were in all other respects healthy.

The control group, composed by 15 dogs, was selected by colleagues from a private practice in a group of voluntary owners that wanted to submit their dogs for an ophthalmologic examination but which were ophthalmological unremarkable on their clinical history. The inclusion criteria were: that they should not have previously been diagnosed with CAD or any other systemic or ocular disease; only one of the animals of the control group

was receiving phenobarbital for two weeks which was considered to not interfere in ocular or skin health.

2.2. METHODS

2.2.1. *Ophthalmological exam*

The ophthalmological exam was performed with the following order: identification of the animal: name, breed, age and gender; anamnesis and collection of a history of ocular symptoms of ocular disease through use of a small questionnaire (Appendix I): the questionnaire sought to document the presence of signs that can be related to the presence of ocular pruritus such as: rubbing the eyes against sofas, carpets, other objects as well as against the owner's legs; and attempts to scratch and rub the eyes with the paws.

The animals were observed on the examination table; instructions regarding proper handling of the animal were given. The ophthalmological exam started with the general examination of both eyes within a distance of 1 meter followed by external examination of the globe and adnexae with a Finoff transilluminator. A Schirmer tear test 1 was performed: the filter paper strip was placed on the inferior conjunctival cul-de-sac, guaranting its contact with the cornea; it was left for 1 minute and the value of tear wetting was measured immediately; with normal reference values being 15-25 mm/min (Ollivier et al., 2007). The next step was the evaluation of ocular reflexes, namely; menace test; palpebral reflex; Dazzle reflex with the slit-lamp; and pupillary direct and indirect light reflexes with the slit-lamp. A drop of oxybuprocain 0.4% solution was placed in ocular surface of both eyes and then the bulbar and external surface of the nictitans membrane was inspected with an atraumatic forceps (fig. 4). This was followed by the observation observation of the anterior segment with the slit-lamp including the evaluation of the presence of Tyndall effect and the realization of the fluorescein test: placing of a fluorescein strip moistened with one drop of NaCl 0.9% in the dorsal bulbar conjunctiva (in non-compliant animals, the impregnated strip of fluorescein was diluted in sterile saline 0.9% and 1-2 drops were applied in the ocular surface); the eye was flushed with sterile saline 0.9% after approximately 1 min. and the corneal surface was inspected with the blue light of the slit-lamp. The administration of a drop of oxybuprocain 0.4% was repeated in each ocular surface and followed by the measurement of intra-ocular pressure with the TonoPen XL (only results with a coefficient of variation < 5% were accepted) and the considered reference range was 15-25 mm Hg (Ollivier et al., 2007).

In all animals with normal values for intra-ocular pressure, a drop of 1.0% tropicamide solution was administrated in both ocular surfaces. The dog was kept in a darkened room for 20 min. and then observation of the lens and anterior vitreous with the slit-

lamp was performed, followed by the observation of the ocular fundus with the indirect ophthalmoscope and with a 20 D lens or with the direct ophthalmoscope - in those animals where we did not have compliance from the owners to dilate the pupil or in those where the value of the intra-ocular pressure was superior to 25 mmHg (even though with no clinically identifiable signs of glaucoma).

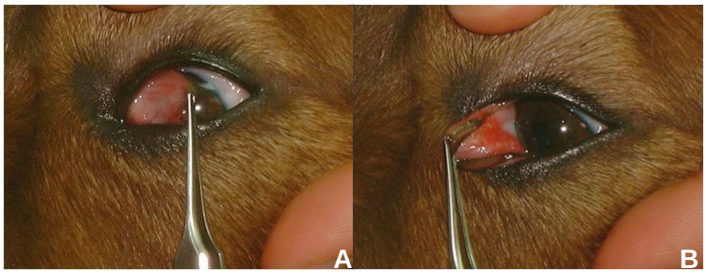


Figure 4 (A and B) – Inspection of the nictitans membrane. A – Inspection of the external surface. B – Inspection of the bulbar surface where it is observed a follicular conjunctivitis.

2.2.2. *Dermatologic exam*

The dermatologic examination was performed in a naturally lighted consulting room by a Diplomate of the European College of Veterinary Dermatology. The patient was examined on the examination table with the help of a student. The exam was performed by systematically examining the skin of the head, neck, dorsum, tail, lateral aspect of the limbs followed by the ventral surface of the body, medial aspects of the limbs, footpads and nails. Finally the ears were examined with an otoscope and appropriate size cone.

3. RESULTS

Study is composed by 30 dogs (n=30) divided in two groups: group A – dogs with atopic dermatitis (n= 15) and group C – control group (n= 15) included by the criteria defined in paragraph 2.1.2. The level of significance utilized for statistical significance was 95% (α=0.05).

3.1. BREED

Using a descriptive analysis of the data, purebred dogs represented 66% (10/15) of the group A and 54% (8/15) of the individuals of the group C. The aim was to compare the frequency of purebred individuals between the two groups. The inferential analysis by Fisher's test, describes an equal distribution between the two groups (p= 0.71) therefore we concluded that both groups are similar in which respects to the presence of purebred dogs.

The most common breeds represented in group A were the French Bulldog (2/15) and the Labrador Retriever (2/15); the other breeds represented in this group were: Alentejo Mastiff, Cocker spaniel, German shepherd, Malinois and Poodle. In C group, the most represented breed were the French Bulldog (2/15) an others breeds represented in the group were: Golden Retriever, miniature Poodle, Peruvian Naked Dog, Poodle and Yorkshire terrier.

3.2. AGE

The age average in group A was 3.5 years (+/- 3.7) and with a range of 1 – 14 years. In C group, age average was 7.3 years (+/-4.1), with a range of 1-16 years. The comparison between the age average between groups was made by Mann-Whitnney (p= 0.007). This analysis shows that the average of ages between the groups was different, being the group A youngest than group C.

3.3. GENDER

Male dogs represented 40% (6/15) of the group A and 80% (12/15) of the individuals of the group C; female dogs represented 60% (9/15) of the group A and 20% of the individuals of the group C (3/15). To compare if there were differences of gender between the two groups a Fisher test was performed (p= 0.06); the differences are not statistically significant but we observed more males in the control group.

3.4. ENTIRE AND NEUTERED ANIMALS

We observed that entire dogs represented 53% (8/15) and 47% (7/15) in the two groups. Fisher test confirmed that the distribution was not significantly different (p= 1.000) in both groups of entire and neutered individuals.

3.5. HISTORY OF OCULAR MANIFESTATIONS OF DISEASE RECOGNIZED BY THE OWNERS IN GROUP A

Through use of the questionnaire mentioned in Materials and Methods (Appendix I) the owners were shown to recognize the presence of ocular symptoms in only 40% (6/15) of the dogs in group A. In the C group, only 6% (1/15) had ocular signs (in this case, chronic epiphora in one eye) which were recognized by the owner after being submitted to the questionnaire previously mentioned.

3.6. OCULAR DISEASE AND HISTORY OF OCULAR SYMPTOMS IN GROUP A

The history of ocular disease in group A was compared with that in group C. The owner noted an ocular problem when asked in 40%; 6/15 of the animals in which an ophthalmological exam was made. In the individuals where the owner was not able to recognize any ocular symptom, 67% (6/9) had ocular disease diagnosed through the ophthalmological exam and only 33% (3/9) did not have any ocular disease. Fisher's test suggests that there are not differences statistically significant between the history and the presence of ocular disease in CAD through ophthalmic examination (p= 0.229).

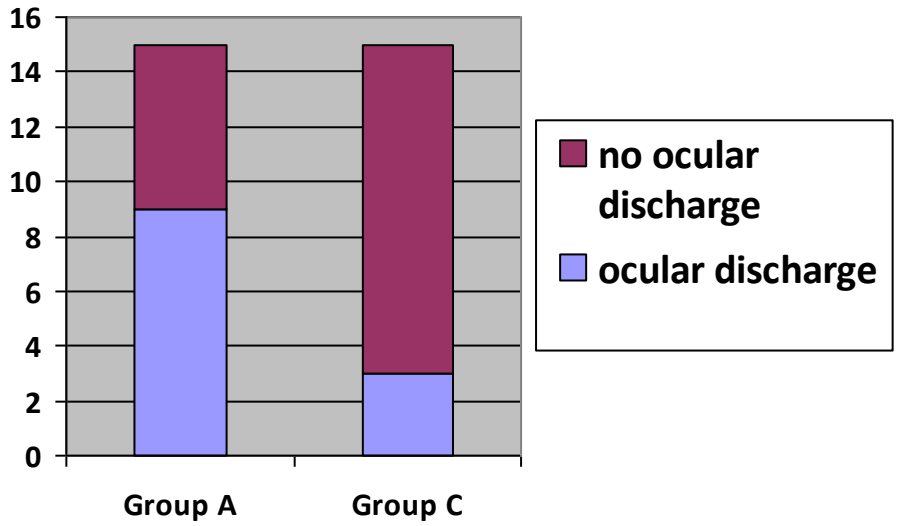
3.7. BLEPHARITIS

Both groups were evaluated for the presence of blepharitis. In group A, blepharitis was diagnosed in 20% (3/15) of the sample; in group C, no animal was diagnosed as having blepharitis. The Fisher's test did not reveal any statistical significance between the presence of blepharitis in the two groups (p= 0.224). In this study is not possible to establish a association between the presence of this ocular disease and CAD presumable because of the small number of animals in the study.

3.8. OCULAR DISCHARGE

The two groups were evaluated for the presence of ocular discharge; in group A, ocular discharge was clinically identifiable in 60% (9/15) of the individuals and it was not present in 40% (6/15) of the individuals; in group C, only 20% (3/15) of the individuals had ocular discharge in the clinical examination; 80% (12/15) were free of this symptom (graph 1). Fisher's test did not reveal any statistical significance between the presence of ocular discharge in the two groups (p= 0.06). Although it was not possible to prove a relation between presence of ocular discharge and CAD, probably because of the small size of the population, this symptom was twice as common in atopic dogs as it is shown in graphic 1.

Graph 1 - Ocular discharge in group A and group C.



In both groups, a mucous discharge was more represented: in group A, where mucous discharge was present in 67% (6/9) of the dogs with ocular discharge; 2 individuals had epiphora and 1 a purulent discharge; in group C, the mucous discharge was also predominant occurring in 67% (2/3) of the dogs with ocular discharge; the other presented with unilateral epiphora; these animals had not any other ocular symptoms or signs of ocular disease; the presence of ocular discharge was, in all cases, minimal and possibly justified by anatomical anomalies related to the drainage of lachrymal fluid in the dog presented with epiphora and, in the others, excessive production of mucous or drainage difficulties. No further examination of the lachrymal apparatus was performed.

3.9. CONJUNCTIVITIS

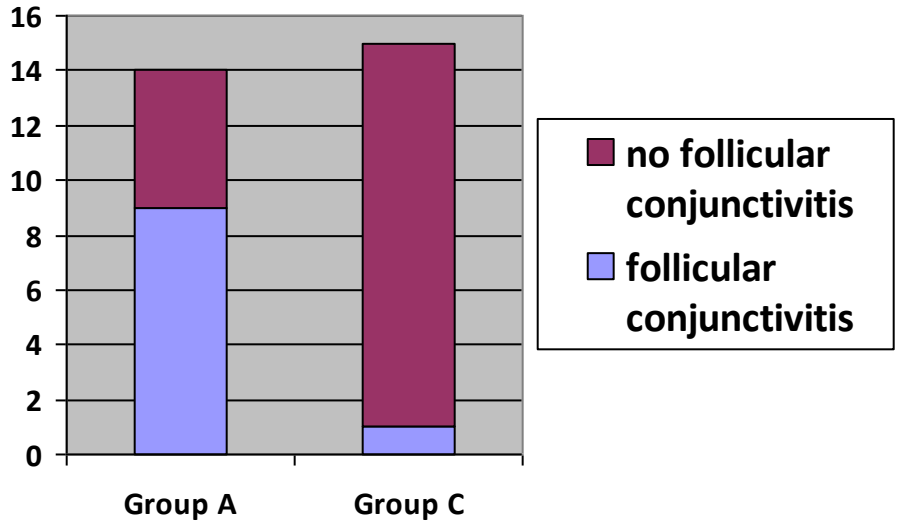
Both groups were evaluated for the presence of conjunctivitis: in group A conjunctivitis was diagnosed in 20% (3/15) of the sample; in group C, 6% (1/15) had

conjunctivitis. Fisher's test did not reveal a strong statistical significance between the presence of conjunctivitis in the two groups (p= 0.598). It was thus not possible to establish a correlation between the presence of conjunctivitis and CAD because of the small sample size, although three times as many dogs had conjunctivitis in the CAD group as in the control group and this difference neared significance.

3.10. FOLLICULAR CONJUNCTIVITIS IN THE BULBAR SURFACE OF THE NICTITANS MEMBRANE (AT LEAST IN ONE EYE OF THE INDIVIDUAL)

Was examined the bulbar surface of 14/15 animals in group A; one animal was non-compliant with this exam. In group A, follicular conjunctivitis in the bulbar surface of the NM was diagnosed in 64% (9/14) of the sample, at least in one eye of the individual; in group C, 7% (1/15) had follicular conjunctivitis in the bulbar surface of the NM at least in one eye. Fisher's test revealed a difference with statistical significance between the two groups (p= 0.002). We concluded that the presence of follicular conjunctivitis occurs more frequently in group A than in group C and can be associated with the presence of CAD.

Graph 2 – Presence and absence of follicular conjunctivitis in group A and group C.



3.11. DISTRIBUTION OF FOLLICULAR CONJUNCTIVITIS OF THE BULBAR SURFACE OF THE NICTITANS MEMBRANE IN GROUP A

The follicular conjunctivitis was present in both eyes in 77% (7/9) of the individuals and was unilateral in 22% (2/9), with 50% (1/2) in the right eye and 50% (1/2) in the left eye. When occurring in both eyes, the severity was similar in 71% (5/7) of the cases and different in 28% (2/7) of the individuals; in one individual was more severe in the right eye and in the

other individual, more severe in the left eye. Follicular conjunctivitis seems to affect both eyes, although, in some cases, the severity may vary between one eye and the other.

3.12. FOLLICULAR CONJUNCTIVITIS AND AGE

The total sample (n= 29) (1 of the animals, as previously mentioned, showed non-compliance for the clinical inspection of the nictitans membrane) was divided in animals having follicular conjunctivitis (at least in one eye) and animals without follicular conjunctivitis. The individuals were also divided by ages: ≤ 18 months (young) and > 18 months (adult). In the young group, 62% (5/8) were diagnosed with follicular conjunctivitis and in the adult group, follicular conjunctivitis was diagnosed in 24% (5/21). The Fisher's test was applied (p= 0.083) and no statistical association was found between the presence of follicular conjunctivitis and young age. It is possible that the lack of association was due to the small size of the sample since follicular conjuntitivis was present three times more frequently in the younger group. It is very likely that, with a larger sample, we could find significant values of p.

3.13. LENS ALTERATIONS

Lens alterations were found in 21% (3/14) individuals in group A; in 14% (2/3) of these animals the presence of cataracts was identified and 7% (1/3) had nuclear sclerosis of the lens. In the C group, 20% (3/15) had lens alterations all corresponding to nuclear sclerosis of the lens. The mean age of the animals with lens alterations is 10.5 years suggesting that they may represent ocular age-related changes.

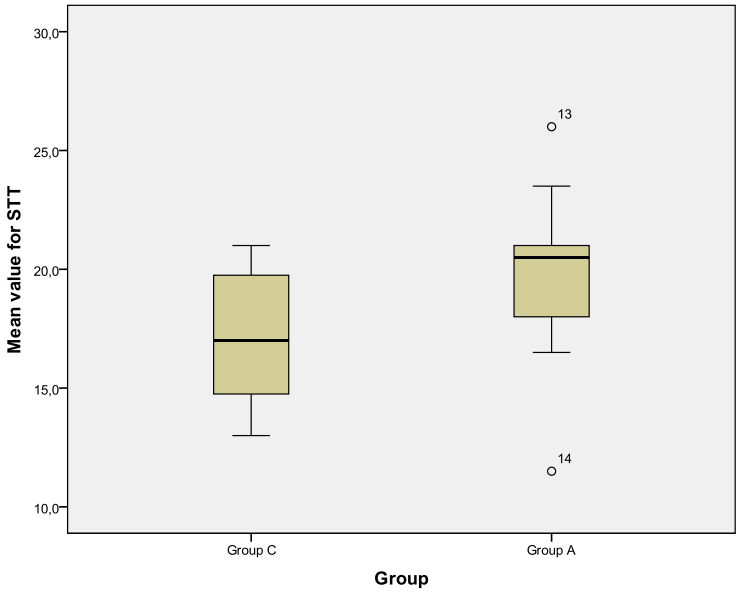
3.14. UVEITIS AND VITREOUS AND OCULAR FUNDUS ALTERATIONS

In the total sample n= 30, we have not identify any alteration compatible with the presence of uveitis, although in two animals was not possible to measure the intra-ocular pressure due to non-compliance of the individual. In the total sample (n= 30), vitreous and ocular fundus observation was possible in 29 individuals and we have not observed any alteration. In the two animals previously described with cataracts was possible a clear view and observation of the vitreous and fundus, since the cataracts were very small (above 1-2 mm); one was subcapsular anterior and the other capsular anterior, both occurring only in the left eye of both individuals.

3.15. VALUES OF SCHIRMER TEAR TEST IN BOTH GROUPS

As a part of the ophthalmologic exam, a STT was performed in 29 animals (one animal of the group A was non-compliant to the performance of this test so it was excluded from this analysis). We have observed that in both groups the results of the STT were within the normal range (5-10 mm/min): 85% (12/14) of the A group had normal values against 14% (2/14) with subnormal values in the group A; group C revealed 80% (12/15) of the individuals normal results against 20% (3/15) with subnormal values. The mean value of STT for the atopic group was 19.93 mm/min (+/- 3.38) and for the control group was 17.26 mm/min (+/- 2.65). We've verified that the applicability of the t-student test was respected and the T-student test to compare means revealed a *p*= 0.025, demonstrating that the mean STT value is higher in the dogs with CAD (graph 3).

Graph 3 – Average of tear production by STT in the patients with CAD and in the control group.



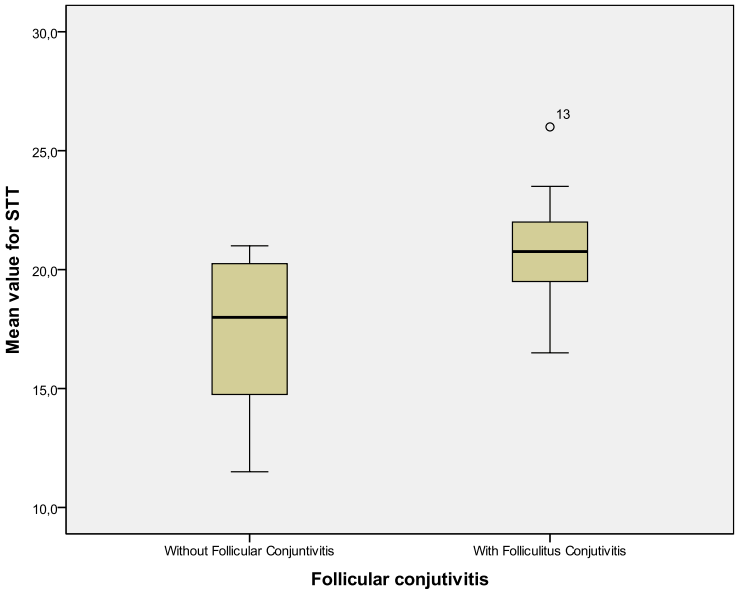
3.16. INTRA-OCULAR PRESSURE IN BOTH GROUPS

The values of intra-ocular pressure in the group A were decreased in 33% (4/12), normal in 50% (6/12) and increased in 16% (2/12) of the individuals. In the C group, the values were decreased in 42% (6/14), normal in 42% (6/14) and increased in 14% (2/14) of the individuals. The mean values of intra-ocular pressure obtained in group A was 18.0 mm Hg (+/- 6.34) and in group C 17.71 mm Hg (+/- 5.81). The means of both groups were compared using t-student test and the value of *p*= 0.9 showing that there are no differences between the two groups.

3.17. FOLLICULAR CONJUNCTIVITIS AND RESULTS OF THE STT

At this point, we aimed to compare the production of tears with follicular conjunctivitis using a descriptive analysis of the data. For the purpose, we defined a sample of 29 patients (n=29); one animal was excluded since the observation of the bulbar surface of the nictitans was not possible due to lack of compliance). This sample was divided in two groups: with follicular conjunctivitis (34.5%; 10/29) and without follicular conjunctivitis (65.5%; 19/29). An animal was considered to have follicular conjunctivitis if at least one eye was involved. In the patients with follicular conjunctivitis, the mean value of STT was 20.8 mm/min (+/- 2.68). The mean value of the STT for the eyes without follicular conjunctivitis was 17.4 mm/min (+/- 2.95). We've verified that the applicability of the t-student test was respected. This test revealed a *p*= 0.005, demonstrating that the mean STT value is higher in the dogs with follicular conjunctivitis. We concluded that in average the tear production is higher in dogs with follicular conjunctivitis.

Graph 4 – Average of tear production by STT in the patients with follicular conjunctivitis and within the patients without follicular conjunctivitis.



4. DISCUSSION

The study group consisted of 66% purebred dogs, being the most representatives the French Bulldog and Labrador Retriever; classically, in CAD, is described a breed predisposition; the breeds that we found more prevalent as having CAD were in agreement to previous studies as predisposed (Sousa & Marsella, 2001; Tarpataki et al., 2006); interestingly, one particular breed, the Alentejo Mastiff is not described as being predisposed to CAD, probably because we lack studies on prevalence of this disease in portuguese breeds.

Our study group sample had more females (60%) then males (40%) representing a minor difference; when comparing to the control group, the presence of females and males were similar, with no statistical significant difference, although the control group had more males (80%) than females (20%). This later might be due to the preference from the owners, in the area where we've studied the control group, for male dogs, instead of female ones. To our knowledge, it is not described a gender predisposition in CAD (Olivry et al., 2001; Hillier & Griffin, 2001; Favrot et al., 2010) so, this difference between the groups is very unlikely to affect our results.

Our study showed no significant difference in prevalence of entire and neutered individuals between the two groups; no clear hormonal influence has been shown in the development of CAD, although it is well known, in human medicine that the hormones can affect the development of some manifestations of atopy, particularly in vernal conjunctivitis which occurs more frequently in young boys in their first decade of life (Bonini et al., 2000; Leonardi et al., 2006).

Dogs in the CAD group were significantly younger (3.5 +/- 3.7 years) than the control group (7.3 +/- 4.1 years). CAD is more prevalent in young animals (with typically development between 1 and 3 years) (Marsella & Girolomoni, 2009) - so it was expected that the mean age would be lower in group A compared with the control group; as described in materials and methods, the individuals were chosen by the colleagues in terms of clients with more known compliance and care for their animals, ensuring the absence of systemic diseases and ocular diseases; the author did not interfere with the choice of sample animals; probably, but the older clients having, naturally older dogs, may have influenced the choice of animals. The fact that owners were content to have their animals undergo an ophthalmic examination may also have influenced the mean age of the control group. Thus although the mean age of the control group (group C) does not match that of the study group (group A), this might not influence the results, since older dogs are less predisposed for the

development of atopy, while youngest ones, can probably show ocular allergy lesions even before they are diagnosed with CAD.

All the dogs were presented for the consultation by their owners; so, the recognition of clinical signs by the owner is a major factor influencing veterinarians to perform a complete ocular examination or to refer the animal for an ophthalmology specialist. None of the dogs of the study group, as mentioned in materials and methods, was presented because of their ocular complaints; but when questioned regarding ocular symptoms, 40% of the owners stated that they had noted at least one ocular symptom (majorly ocular discharge); where the owner noted an ocular symptom their recognition was shown to be correct on ophthalmic examination which identified ocular disease. On the other hand in animals shown to have ocular disease by ophthalmic examination owners had not recognized an ocular symptom in 67% of the animals. This fact might be explained through the difficulty owners had in recognizing mild signs of ocular disease, such as, for instance, a slightly redness of the conjunctiva or a follicular conjunctivitis of the bulbar surface of the nictitans membrane. Another reason might also be the fact that no dog, in this study, had severe ocular disease - most of the lesions were of mild or minor severity. To our knowledge, no study has to date compared the recognition of clinical signs by the owner with those diagnosed by the veterinarian in atopy related ocular disease, although Lourenço- Martins et al. (2011) describe ocular lesions related to atopy as being misdiagnosed by the dermatology service in 72% of the allergic conjunctivitis cases, supporting our theory that a more specific ophthalmologic exam is necessary in order to evaluate and diagnose correctly the patients in which ocular manifestations are associated with CAD.

The ocular symptoms of CAD such as blepharitis, blepharoconjunctivitis, follicular conjunctivitis and allergic conjunctivitis have been described by several authors (Bedford, 1999; Hendrix, 2007; Stades & Gellat, 2007; Peña & Leiva, 2008; Lourenço-Martins, 2011). To our knowledge, none has described the clinical ocular presentation of dogs with CAD which had not received any treatment for atopy. Lourenço-Martins et al. (2011) describe a variety of clinical signs of ocular allergy but prevalence of the different ocular diseases associated to CAD is not described; by the other side, is not referred, in their study, if the patients were on treatment for CAD at the time of ocular examination. In our study group (group A), a high prevalence of 20% (3/15) of blepharitis, 20% of conjunctivitis (3/15) and 64% (9/15) of follicular conjunctivitis of the nictitating membrane was detected. When compared to the control group, only the increased presence of follicular conjunctivitis showed statistical significance (p= 0.02) allowing us to suggest an association of this disease with CAD. With regard to the other ocular diseases previously mentioned as being associated to

CAD, we could not establish a statistically significant association while comparing group A with group C; but it must be recognized that our sample for each group was small (n= 15) and more cases should be added, in the next future, to this study in order to determine whether a significant association exists. For instance, the presence of ocular discharge and CAD, was very close to significance since our *p* value ($p = 0.06$) for this correlation was very close to the achievement of a $p < 0.05$. Regarding to the presence of conjunctivitis was not also possible to prove a correlation between this disease and CAD, although we've found three times more cases (3/15) in group A than in group C (1/15) as well as for blepharitis.

In human medicine, the presence of conjunctival papules seems to be present within all the different manifestations of ocular atopy (Donshik, 1994; Bonini et al., 2000; Bonini, 2004; Bielory & Bielory, 2010) with exception to seasonal and perennial allergic conjunctivitis (Bonini, 2004; Stahl & Barney, 2004; Leonardi et al., 2008); these papules, even though clinically might be very similar to the hyperplastic follicles seen in the dog, have a different constitution and not described as being part of the Conjunctival associated lymphoid tissue (CALT) (Belfort et al., 2000; Bonini et al., 2000); The blepharoconjunctivitis that occurs seems to be associated with ocular pruritus, since there is auto-infliction and disruption of the skin barrier which may lead to trauma and secondary infections (Bistner, 1994; Whitley, 2000). On the other hand the follicular hyperplasia observed seems to be a local response to antigens (Peña & Leiva, 2008) and, at least to our knowledge, in the dogs, is has not been correlated with the presence of ocular pruritus. The ocular/periocular pruritus that dogs show in CAD might be due to dermatologic pruritus of the periocular skin or due to allergic conjunctivitis and it is very difficult to distinguish either one or the other as the focus of irritation. To our knowledge, a specific and understandable cause of ocular pruritus associated with CAD has not been yet described.

The presence of follicular conjunctivitis almost always occurred in both eyes (n=7), although 2 of these animals showed different grades of severity between both eyes; in the animals diagnosed with follicular conjunctivitis (n= 9) 2 only showed follicular conjunctivitis in one eye; the different grades of severity found might be explained trough the fact that all animals showed only mild symptoms of CAD; probably, with time and without no systemic control for CAD, it would be expected an aggravation of the associated ocular lesions that could evolve both eyes. A study with a larger population with different grades of severity of CAD and it's correlation with the severity of ocular disease might contribute for the explanation of this theory.

Interestingly with regard to the presence of follicular conjunctivitis, we have not found a direct correlation between this diagnosis and young age (<18 months as previously

described in the literature). This might be due to the fact that, as mentioned in our results, in section 3.10., follicular conjunctivitis can be associated with CAD, probably being an ocular manifestation of this disease, instead of an age predisposed ocular disease. It would be of interest in the future to try to support this hypothesis by studying only populations of atopic dogs with ages inferior to 18 months and comparing the observations with a control group of the same range of age. The lymphoid follicles of the conjunctiva are also considered to be CALT which is thought to be involved in the protection of the ocular surface by performing antigen uptake, processing and initiating immune responses in analogy to other mucosal associated lymphoid tissue (MALT) (Steven & Gebert, 2009). CALT is also described as a MALT inductive site from where T and B lymphocytes migrate to effector sites; these effectors sites being present in all mucosal tissues as lymphoid tissue diffusely distributed throughout the lamina or substantia propria (Cesta, 2006). The functions of CALT may support our theory that follicular conjunctivitis, due to overgrowth of the lymphoid follicles, might be an ocular manifestation associated to CAD, probably, an incipient one that might occur earlier than other ocular manifestations.

The presence of ocular discharge was found to be more common within the group of study than in the control group; even though a statistical significance was not demonstrated, the *p* value obtained (p= 0.06) suggests that, with the enlargement of the sample size, the presence of ocular discharge might be associated with CAD. Since the ocular discharge is present as a symptom in 78% of the individuals having follicular hyperplasia, it might be a relevant clinical sign for the clinician to observe of the bulbar surface of the nictitans membrane in dogs with CAD and to submit them to an ophthalmological exam; the presence of follicular lymphoid hyperplasia in this anatomic localization has been associated mostly to the presence of chronic conjunctivitis (Ollivier et al., 2007) being typically associated with an ocular mucoid discharge (Bedford, 1999; Hendrix, 2007). On the other hand, even though the most animals had results of the STT within normal values, animals with follicular conjunctivitis and CAD had a higher mean STT compared to the animals without this disease. The mean value of STT was 20.8 mm/min (+/- 2.68) in the dogs with follicular conjunctivitis, and was 17.37 mm/min (+/- 2.95) in the dogs without follicular conjunctivitis. The obtained *p* value (0.005) while comparing these two groups proves that the values of the STT are significantly higher in the dogs having follicular conjunctivitis. This might be due to mechanical irritation to the bulbar conjunctiva of the globe by the presence of the follicles causing an anomaly of the bulbar surface of the nictitans membrane, stimulating an additional production of the aqueous part of the tear film thus causing an increase of the STT average values in the affected animals, or might be part of the irritative focus which is

causing the follicular conjunctivitis concurrently. The CAD group had also significantly (p= 0.025) a higher mean value of STT while comparing to the control group, also suggesting that dogs with CAD can have higher lachrymal production (aqueous layer) while comparing to normal dogs; in the meanwhile we have to interpret this results knowing the fact that the control group had significantly older animals which may have influenced this difference between the two groups.

5. CONCLUSIONS

This study supports our presupposition that dogs with CAD commonly have associated ocular disease thus justifying the need of every dog with atopic dermatitis to undergo an ophthalmological exam.

In the atopic dogs, the most prevalent ocular sign was the presence of follicular conjunctivitis in the bulbar surface of the nictitans membrane this type of conjunctivitis was associated to the presence of CAD in our study group allowing to the conclusion that it might represent a manifestation of ocular allergy associated with CAD instead of an ocular finding only related with younger ages (< 18 months) as described in the literature.

In some atopic dogs, the presence of blepharitis and conjunctivitis was also detected, but a true relation between these diseases and CAD in our study group was not established; interestingly a much lower prevalence of blepharitis and conjunctivitis was detected when compared with that described in the literature (Lourenço-Martins, 2011). This might correlate with the fact that the commonly described blepharoconjunctivitis is more a secondary manifestation due to periocular skin pruritus and subsequent skin and conjunctiva infection by disruption of the skin barrier (as seen in pyoderma associated in CAD) than a primary ocular manifestation of CAD.

The ocular manifestations of disease reported from the owners, mainly ocular discharge, always corresponded to the presence of ocular disease; but on the other hand, in 67% of the cases with ocular disease, the owners did not report any ocular symptom. This emphasizes, again, the need of performing an ocular examination as a complementary exam in all cases of CAD.

Further prospective studies with a larger population in both groups are needed with the aim properly to classify and describe unique ocular manifestations of CAD, as already occurs in human medicine. The complement of the ocular examination with additional laboratory exams such as conjunctival cytology of both conjunctivas: eyelids and bulbar surface of the nictitans membrane and measurement of lacrimal IgE as well as the provocation conjunctival test described by Lourenço-Martins et al. (2011) in all affected animals would contribute to the understanding of the pathophysiology of the ocular disease in CAD and to the establishment of a better documented ocular syndrome associated with CAD.

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APPENDICES

Appendix I – Questionnaire to the owners.

Question 1 – has your dog ever been diagnosed of any king of ocular disease?

Question 2 – is your dog receiving any ocular or systemic medication?

Question 3 – does your dog appear normal to you relating to the aspect of the eyes?

Question 3 – does your dog have or have been having in the past any kind of ocular discharge? If so, what kind of ocular discharge do you recognize? Transparent? White? Green? Yellow?

Question 3 - does your dog rub its paws in the face or periocular area?

Question 3 – does your dog rubs its eyes or face against carpets, sofas, or even your legs?

Question 4 – does your dog, in any time, appear to you has having red eyes?

Question 5 – does your dog, in any time, appear to have the eyes more closed than the normal? Only in one eye or both?

Question 6 – have you notice any abnormality relating to your dog's vision?